REMARKS

Applicant has amended the specification, paragraphs [051] and [052] to delete hyperlinks. The amendments are clerical and thus do not introduce new matter. Entry of the amendments is respectfully requested.

Applicants have amended claims 1, and 9 step (b), and claims 12 and 17, step (c) to make explicit that which was implicit, namely that the at least two primer pairs are used in the same amplification reaction, i.e., in a multiplex manner. The amendment is supported throughout the specification, for example at paragraph [016]. Applicants have further amended claims 1 to prefect the dependency, and refer to single molecule dilution which is made in the step (a) rather than single nucleotide dilution. The amendment is supported throughout the specification, for example at paragraph [016].

Applicants have further amended claim 1, step (c) to explicitly mention that the two nucleic acids regions amplified in step (b) contain at least one polymorphic marker each. The amendment is supported throughout the specification, for example, in paragraph [015].

Applicants have amended claims 4 and 6 to perfect the dependency. These amendments are clerical.

Applicants have amended claim 7 to remove the multiple dependencies. As such, the amendment is clerical.

Applicants have further amended claim 17, step (e) to correct a grammatical error, when referring to "at least one marker." This amendment is also clerical.

Applicants have added claim 19. This amendment is supported by the paragraph [065] and the examples.

Accordingly, the amendments do not introduce new matter and their entry is respectfully requested.

The Examiner objected to the specification because it contained references with hyperlinks. In light of the amendments to the specification, *supra*, Applicants respectfully request that the objection be withdrawn.

The Examiner objected to claim 17 because of the grammatical error in the phrase "at least one markers." In light of the amendment to claim 17, *supra*, Applicants respectfully request that the objection be withdrawn.

The Examiner objected to claim 7 due to multiple dependency. In light of the Amendment to claim 7, *supra*, Applicants respectfully request that the objection be withdrawn and claim 7 examined on the merits.

Claims 1-6 and 8-11 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

Specifically, the Examiner contended that there is no antecedent basis for the phrase "the diluted single nucleotide dilution."

Applicants have amended the claims as shown, *supra*, to make explicit that which was implicit, namely, that the reference is made to the single molecule, i.e., nucleic acid molecule, as referred to in the step of making a single molecule, i.e. single nucleic acid molecule dilution. Accordingly, Applicants respectfully submit that the rejection be withdrawn.

Moreover, the Examiner contended that the phrase "the polymorphism" in claims 4, 5, and 6 is unclear because claim 1, from which the claims depend, recites to multiple polymorphisms. In light of the amendments, *supra*, Applicants respectfully request that the rejection be withdrawn.

In light of the above, Applicants respectfully submit that the claims now comply with 35 U.S.C. §112, second paragraph.

Claims 1, 2, and 4-6 were rejected under 35 U.S.C. §102(b) as being anticipated by Ruano et al. (1990, as cited in the IDS) ("Ruano").

Applicants respectfully disagree and submit that this rejection should be withdrawn for the following reasons.

Applicants respectfully submit that, as stated in the background of the invention in par. [004] Ruano is different from the presently claimed methods, and does not teach all the elements of claims 1, 2, and 4-6.

Specifically, the Examiner contended that Ruano teaches using amplification of the target DNA with two different primer pairs (GR1, GR3, and GR2, GR4). Applicants respectfully disagree. Ruano discloses amplification of one region of the DNA template using only one primer

pair, namely GR5/GR6 (see, Figure 1). Primers GR1/GR3, and GR2/GR4, are allele specific amplification primers (ASO, as taught in, e.g., Fig. 1 of Ruano), and thus not used as primer pairs to amplify a region which contains the polymorphisms, as required by claim 1. Rather, these primers are used for detecting whether an amplified template has a specific polymorphism, and thus, for example, either primer GR1 **or** primer GR3 results in amplification, of the specific allele in the TG deletion site within the specified TG repeat, which constituted on of the polymorphisms detected in Ruano. Thus, Ruano's method differs from the present one in that Ruano only shows genotyping and haplotyping within one template at a time.

In contrast, the present invention is directed to a method for determining a haplotype using **multiplexing**. As explained in the specification "multiplexed reactions" refer to having at least two different sets of extension primers in the same reaction. There can be a large number of such reactions going on simultaneously. The present method requires use of at least two primer pairs to **simultaneously** amplify at least two regions in the template DNA in a multiplex manner. Claim 19 requires the use of at least four primer pairs. The Examiner contended that the Ruano discussion mentions that "distant segments of an intact template molecule can be analyzed by PCR with multiple primer pairs for direct haplotyping" (page 4, par. 8, second to last sentence of the 4/12/2006 Office Action). Ruano discussed that:

> "[f]or haplotype determination of sites separated by a distance exceeding the current limits of routine PCR (~3 kb), **separate** SMD amplifications of overlapping regions could allow logical reconstruction of the extended haplotype based on one allele shared at a heterozygous site within the overlap." (p. 6300, left col. 1st par. of the Ruano Discussion, emphasis added).

Thus, what Ruano taught the skilled artisan was use of **separate** amplification reactions, not a single simultaneous multiplex analysis, i.e., one where multiple reactions are occurring in the same reaction. In the next sentence after the above reproduced quote, Ruano contemplates that an overlap of the fragments may not be needed. Ruano simply does not disclose use of multiplex analysis of multiple parallel dilutions in a single reaction, as required by the present claims. The present specification discusses, for example, that you can have simultaneous genotyping of several different markers, see paragraph [042].

Accordingly, in light of the above, Applicants respectfully request that the rejection of claims 1, 2, and 4-6 were rejected under 35 U.S.C. §102(b) under Ruano be withdrawn.

Claims 3 and 9-11 were rejected under 35 U.S.C. §103 as being obvious over Ruano in view of Drysdale et al. (2000, as cited in the IDS) ("Drysdale").

Applicants respectfully disagree, and submit that the rejection be withdrawn for the following reasons.

As discussed, *supra*, Ruano does not teach all the elements of claim 1 or 2, upon which claim 3 depends, namely, multiplex amplification. With respect to claim 9, as discussed, Ruano does not teach or suggest the use and advantages of multiple reactions simultaneously, i.e., multiplex amplification. Drysdale does not overcome this deficiency. All Drysdale discloses is that one can compare the resulting haplotype with another. Nothing in Drysdale teaches or suggests using a multiplex amplification of single molecule dilution to resolve haplotypes.

In light of the above, Applicants respectfully submit that the rejection of claims 3 and 9-11 under 35 U.S.C. §103 over Ruano in view of Drysdale be withdrawn.

Claim 8 was rejected under 35 U.S.C. §103 as being obvious over Ruano.

Applicants respectfully disagree, and submit that the rejection be withdrawn for the following reasons.

As discussed, *supra*, Ruano does not teach or suggest using multiplex amplification in a single reaction, as required by claim 1. Accordingly, Ruano does not teach all the elements of claim 8, and the rejection should be withdrawn.

Moreover, while one may wish to increase accuracy, one would have to balance it with efficiency. There must be a reason why the artisan would be motivated to increase the repeats to 12-18, rather than 2 or 5 as disclosed by Ruano. Ruano specifically discusses, that the ambiguities that are left by the method, while reducing efficiency, **do not affect accuracy** (p. 6300, left lane, 2nd full par.). Thus, if anything, the artisan reading Ruano, would come to the conclusion that 2 to 5 repeats are enough for accurate results, and no additional repeats would be needed. Applicants teach specifically, that the increase in the repeats increases the efficiency. For example in par. [077], the specification discloses that increasing the repeats from one to 4 increases the efficiency from about 40-45% up to about 90%. The specification also discusses the finding that about 5-10% of the multiplex genotyping calls are incomplete, and that replicas for the present analysis

were typically preformed about 12-18 times to increase efficiency. Thus, Applicants respectfully submit that without reading the reasons for how much one can actually increase the success in the analysis, one would not have been motivated to increase the number of assays.

Accordingly, Applicants respectfully request, that Ruano neither teaches nor suggests claim 8 and thus, the rejection of claim 8 under 35 U.S.C. §103 over Ruano should be withdrawn.

Claims 12-18 were rejected under 35 U.S.C. §103 as being obvious over Ruano in view of Rein et al. (1998) ("Rein").

Applicants respectfully disagree, and submit that the rejection be withdrawn for the following reasons.

Applicants have amended claims 12 and 17 as described, *supra*. As discussed, *supra*, Ruano does not teach use of multiplex amplification. Rein does not overcome this deficiency because nothing in Rein teaches or suggests the multiplex amplification of the diluted sample as required by claims 12-18.

Accordingly, Applicants submit that Ruano in view of Rein neither teaches nor suggest the invention of claims 12-18, and the rejection under 35 U.S.C. §103 should be withdrawn.

Claims 1-18 were provisionally rejected under 35 U.S.C. §101 as claiming the same invention as that of the co-pending application Serial No. 10/542,043.

Applicants note the rejection, and respectfully request that it be held in abeyance pending allowance of the claims over the other rejections.

U.S. Serial No. 10/759,519
Amendment responsive to 4/12/2006 Office Action
Amendment dated October 12, 2006

In view of the foregoing, Applicant respectfully submits that all claims are in condition for allowance. Early and favorable action is requested.

In the event that any additional fees are required, the PTO is authorized to charge NIXON PEABODY LLP Deposit Account No. 19-2380.

Respectfully submitted,

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